MAGI3–AKT3 fusion in breast cancer amended

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ARISING FROM S. Banerji *et al*. *Nature* **486,** 405–409 (2012)

Banerji *et al.*[1] described a novel *MAGI3–AKT3* rearrangement in breast cancer, enriched in triple negative tumours. The report was highly encouraging as targeted therapies could potentially serve as a new and much needed option to treat this highly aggressive breast cancer subtype. We sought to confirm the presence of this rearrangement in 236 samples of triple negative breast cancer (TNBC) by using fluorescent *in situ* hybridization (FISH) and reverse transcription-polymerase chain reaction (RT–PCR), and in 84 additional cases from The Cancer Genome Atlas by using FusionSeq. No evidence of the fusion was found in any of the tumours studied. Our study confirms that *MAGI3–AKT3* fusion is not a recurrent event in triple negative breast cancer, which should be acknowledged before considering the evaluation of targeted therapies in clinical trials. There is a Reply to this Brief Communication Arising by Pugh T. *et al. Nature* **520**, http://dx.doi.org/10.1038/nature14266 (2015).

TNBC constitutes the majority of breast carcinomas of the basal-like molecular subtype, and is defined by absence of actionable therapeutic targets (ER, PR, HER-2). TNBC patients have a poor response to conventional breast cancer therapies[2] and experience poor survival[3]. As such, molecular elucidation of these tumours is critical in the hopes of developing novel targeted therapies[4].

Discovery of functionally recurrent gene rearrangements is a relatively new avenue in breast cancer (for example, MAST kinase and Notch gene families[5]). Banerji *et al.* reported a *MAGI3–AKT3* gene fusion to be present in 7% (5/72) of TNBC[1]. This balanced translocation results in a constitutive activation of AKT kinase, which can be countered using small molecule AKT inhibitors.

We aimed to determine the frequency of *MAGI3–AKT3* fusion in 236 TNBCs represented in high-density tissue microarrays (see Table 1). Following previously described protocols[6–8], FISH was performed using dual colour locus-specific probes for *MAGI3* and *AKT3*. None of the cases showed either *MAGI3* or *AKT3* break-apart or fusion signals. To exclude the possibility of intra-tumour heterogeneity, multiple regions of full tumour sections were screened in a subset of 28 cases, all of which were also negative for break-apart and fusion signals (see Fig. 1). Additionally, shorter primer sequences were designed to test a 187 bp fusion product of intron 9

of *MAGI3* with intron 1 of *AKT3* in archival material. We performed RT–PCR of cDNA in 135 of these cases. No *MAGI3–AKT3* fusion product was detected. Further, we investigated RNA-seq data from 84 TNBC cases from The Cancer Genome Atlas with FusionSeq[9]. We did not find any evidence of *MAGI3–AKT3* gene fusion in these cases either.

Our sample size has sufficient power to detect with 95% confidence gene rearrangements that would occur at a frequency of as low as 3%. Based on our results, we can reliably conclude that *MAGI3–AKT3* rearrangement is neither recurrent nor sub-clonal in TNBC. To assume that *MAGI3–AKT3* fusion was a recurrent event in TNBC, Banerji *et al.*[1] interrogated their tumours by using RT–PCR of cDNA followed by Sanger sequencing only. Confirmation at the genomic level by PCR of genomic DNA was performed exclusively in the index case. Hence, we favour that, with the exception of the index case, the sequenced RT–PCR products by Banerji *et al.*[1] represent a post-transcriptional fusion event (*trans*-splicing), rather than a true genomic event.

Our patient cohort of mainly Caucasian women in contrast to those of Mexican and Vietnamese decent studied in Banerji *et al.*[1] raises the possibility that this rearrangement may be population-enriched, a prospect that needs further study.

Methods

Locus specific probes were located at 1p (*MAGI3*: BAC 5′ RP11-1133G15 and 3′ RP11-1008I9) and 1q (*AKT3*: BAC 5′ RP11-931B5 and 3′ RP11-989N14). At least 150 nuclei per case were interrogated in tissue microarrays. In full sections, ~2,000 nuclei per slide were evaluated. FusionSeq[9] is a robust computational tool to detect fusion transcripts in paired-end RNA-seq data[10–12]. Reads were aligned to the human reference genome sequence (GRC37/hg19) using STAR[13]. PCR primers sequences are as follows. *MAGI3* forward: 5′-TGTCCTTGTTCGAGCATCAC-3′, *MAGI3* reverse: 5′-GAGGACACAGTTGCCATTGA-3′, *AKT3* forward: 5′-TGAAAGAAGGTTGGGTTCAGA-3′, *AKT3* reverse: 5′-GCCACTGAAAAGTTGTTGAGG-3′. *PGK* was used as control gene.

Figure 1 Absence of *MAGI3–AKT3* fusion in triple negative breast cancer. a, Haematoxylin and eosin (H & E) stained full section of a representative case of triple-negative breast cancer. **b**, **c**, Tissue microarrays (**b**) and multiple regions of the entire section (**c**) were interrogated by FISH (total of 236 cases). **d**, No break-apart signals for *MAGI3* or *AKT 3* were identified. No *MAGI3-AKT3* fusion product was detected by RT–PCR in a subset of 135 cases.

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Table 1: Clinico-pathologic characteristics of 236 triple negative breast cancers

Patient Age	22–92 years	
Tumour size	0.3–7.4 cm	
Number of tumour type cases†	Invasive ductal carcinoma	218*
	Invasive lobular carcinoma	5
	Metaplastic carcinoma	5
	Other	8
Stage (percentage of cases)	Stage IA	47.1%
	Stage IB	0.6%
	Stage IC	0.6%
	Stage IIA	31.4%
	Stage IIB	6.5%
	Stage IIIA	4.6%
	Stage IIIB	2.0%
	Stage IIIC	4.0%
	Stage IV	2.6%
	Not available	0.6%
Ki-67 (proliferation index	High (≥ 10%)	219 cases
	Low (< 10%)	17

Weill Cornell cohort (*n* = 153) and University Hospital Zürich cohort (*n* = 83)
†Includes 6 cases from recurrence or metastases, as follows: 1 case of chest wall recurrence; 3 cases of ipsilateral lymph node metastases, 1 case of upper arm metastasis, 1 case of femoral metastasis (Weill Cornell cohort)
*Includes two patients who had bilateral tumours (Weill Cornell cohort).

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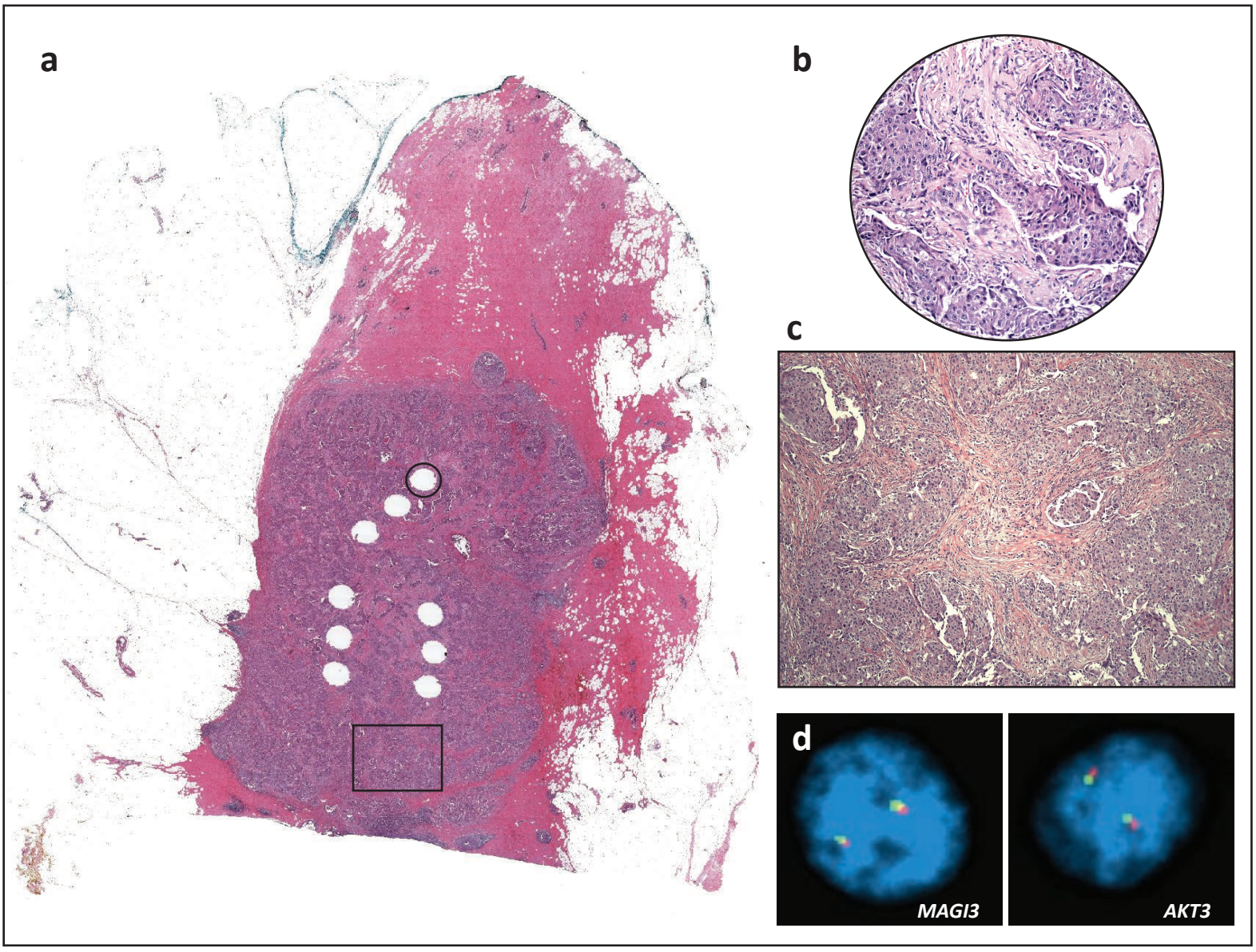
a
b
c
d
MAGI3
AKT3